

media and transgene induction. Although these two methodologies could be combined, it is difficult to find the best combination due to its complexity. Recently, we have developed the NIA mouse ES cell bank: ~100 ES cell lines, each of which carries an inducible transcription factor (TF) based on Tet-Off system. Here we examine how the induction of TFs, coupled with the environmental manipulation, can modulate the pathways of ES cell differentiation (assessed by cell proliferation, morphology, histology, and characterization by microarray). We have tested the effect of TF induction during 2-dimensional cell differentiation that was triggered by LIF withdrawal, retinoic acid (RA) addition or 3-dimensional cell ES cell differentiation by teratoma formation in mouse. Our study revealed that various TF inductions could indeed lead to differentiation of ES cells into specific cell fates. For example, forced induction of Pou5f1 in the presence of RA seemed to differentiate ES cells into neural cell lineage; Rxra induction during teratoma formation made yolk sac like structure that is supposed to be a sort of functional tissue. As these cell types or structures could not be obtained by conventional methods, our systematic approach may provide an efficient means to diversify the cell types that can be differentiated from ES cells.

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Program/Abstract # 386

Investigating piwi function in *Hydra* stem cells

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The Piwi proteins are required for stem cell maintenance in diverse organisms and their molecular functions in this regard are currently under intense investigation. Piwi proteins associate with a distinct class of small RNAs between 26 and 30 nucleotides in length, called piwi-interacting RNAs (piRNAs). Piwi proteins modulate gene expression at both the epigenetic and post-transcriptional levels, and thus appear to have a wide-reaching effect on the gene expression profile of a cell. In the genome of *Hydra magnipapillata* we find two *piwi* orthologs. By fluorescent *in situ* hybridization (FISH) we find that both *Hydra piwi* genes are expressed specifically in the interstitial cells (I-cells), a well-characterized stem cell population that gives rise to both somatic cells and germ cells. Furthermore, we find that *Hydra* has an abundant population of putative piRNAs. Advances in *Hydra* methodology, such as the ability to make transgenic animals, are allowing us to investigate Piwi and piRNA functions in the *Hydra* stem cells *in vivo*. Cnidarian gene sets, such as *Hydra*, exhibit the same complexity found in vertebrate genomes, thus functional discoveries made in these relatively simple metazoans will be applicable to bilaterians.

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Program/Abstract # 387

Serpent, Suppressor of Hairless and U-shaped are critical regulators of hedgehog niche expression and prohemocyte maintenance during *Drosophila* hematopoiesis

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The lymph gland is the larval hematopoietic organ in *Drosophila*. It is composed of three different cell populations: the medullary zone (MZ), the cortical zone (CZ) and the posterior signaling center (PSC). The MZ is comprised of blood progenitor cells, while the CZ consists of mature hemocytes. The PSC is a hematopoietic stem cell niche that

maintains the MZ. Hedgehog (Hh) is a signaling molecule that regulates various developmental phenomena and has known expression in the PSC. In the absence of Hh signaling, prohemocytes are not maintained. To understand how hh gene expression is controlled in the PSC, we have characterized a *hh* enhancer driving PSC-specific hh gene expression. Our results have shown that a combination of positive and negative transcriptional inputs promote proper hh expression. The GATA factor Serpent (Srp) is required for hh expression in PSC cells, while Suppressor of Hairless (Su(H)) and U-shaped (Ush) transcriptional regulators prevent hh expression in blood cell progenitors and differentiated hemocytes. We have also shown that regulation by Su (H) appears to be a Notch-independent process. In addition, it is likely that hh is required for its own self-maintenance. These results suggest a collaboration of the three transcription factors in the regulation of proper hh gene expression and niche cell function.

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Program/Abstract # 388

Establishment of novel chicken embryonic stem cells capable of differentiating into germ cells

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Chickens are an ideal developmental biology animal model, as well as a farm animal with excellent productivity. Therefore, researchers have long sought to establish chicken embryonic stem cells (cESCs) that enable the creation of genetically modified chickens. Unfortunately, after long-term culture, germ cells lose the expression of the germline-specific genes, including chicken vasa homolog (Cvh) and deleted in azoospermia-like (DAZL), as well as their capacity for differentiation. Here, we derived novel cESCs from epiblasts cultured with chicken leukemia inhibitory factor (cLIF). These cESCs have the capacity for long-term successive subculture and express chicken *Nanog*, *Cvh* and *DAZL*. The cESCs showed a capacity for chimeric formation during a transplant experiment that used a fertilized egg. In order to create transgenic chickens using established cESCs, EGFP and puromycin resistance genes were transfected into cESCs. After selection with puromycin, we then transplanted EGFP-cESCs to a recipient embryo of a fertilized egg. EGFP expression was detected in many cells of embryos. The transferred embryo proceeded to develop normally, and chimeric chickens hatched. Expression of EGFP among Cvh positive cells was observed in primordial germ cells. Moreover, we confirmed the presence of EGFP gene in sperm genome of chimeric chickens. These results indicate that novel cESCs have the capacity to differentiate into germ cells.

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Program/Abstract # 389

Patterning of blood vessels is controlled by neural progenitor cells in the central nervous system

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Vascular networks of blood vessels are stereotypically formed in early developing embryos. It remains unknown how the vascular

patterns are established in three-dimensional tissues including peripheral organs and the central nervous system (CNS). We study the mechanisms underlying the patterning of blood vessels in CNS using early spinal cord as a model. In chicken E4 embryos, endothelial cells of somitic origin penetrate into the spinal cord through the ventral edge. Subsequently, forming vessels extend dorsally along determined paths. We found that these paths coincide with the interface between the layer of progenitor cells (medial) and the layer of differentiated neurons and glial cells (lateral). Since the blood vessels do not invade the progenitor layer, we reasoned that the progenitor layer elicits inhibitory influence on the blood vessel formation. To test this, we experimentally promoted neural/glial differentiation in the progenitor territory. When the progenitor layer was electroporated with Pax2, a transcription factor known to promote glial cell differentiation, the cells underwent precocious differentiation. Remarkably, in the Pax2-electroporated region, an ectopic formation of blood vessels was observed. These observations support the idea that the blood vessel patterning in CNS is regulated by local environment, where neural progenitor cells appear to prevent the vessel formation. We will discuss the relationship between vascular patterning and neural differentiation.

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Program/Abstract # 390

Regulation of the CamkII node determines proliferative potential of growth plate chondrocytes

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For tissues that develop throughout embryogenesis and into post-natal life, the generation of differentiated cells to promote tissue growth is at odds with the requirement to maintain the stem cell/progenitor cell population to preserve future growth potential. In the growth plate cartilage, this balance is achieved in part by establishing a proliferative phase that amplifies the number of progenitor cells prior to terminal differentiation into hypertrophic chondrocytes. Upregulation of endogenous calcium/calmodulin-dependent protein kinase II (CamkII) activity in chondrocytes promotes proliferative phase exit and is required for chondrocyte hypertrophy. CamkII activity in chondrocytes is under negative regulation by the Wnt and the Pthrp signaling pathways and release of repression activates multiple independent, dosage-sensitive effector pathways that compose the terminal differentiation (hypertrophic) program. In doing so, CamkII activity places strict limits on the growth potential of proliferative chondrocytes. We present an integrated model for the regulation of proliferative potential that has important implications for studies of adult stem cells.

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Program/Abstract # 392

Epicardial spindle orientation controls cell entry into the myocardium

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During heart morphogenesis, epicardial cells undergo an epithelial to mesenchymal transition (EMT) and migrate into the subepicardium. The cellular signals controlling this process are poorly understood. Here, we show that epicardial cells exhibit two distinct mitotic spindle orientations, directed either parallel or perpendicular to the basement membrane. Parallel division, in which the mitotic spindle is $\sim 180^\circ$ to the basement membrane, results in epicardial cell expansion, while cells undergoing perpendicular cell divisions subsequently enter the myocardium. We found that loss of β -catenin led to a disruption in adherens junctions, randomization of mitotic spindle orientation, and reduced cell entry into the myocardium. Surprisingly, loss of β -catenin correlated with the loss of Numb asymmetric localization to adherens junction and basal domain of epicardial cells. Loss of Numb and Numblake in the epicardium disrupted adherens junctions, led to randomized mitotic spindle orientations, and also had reduced numbers of epicardial cells entering the myocardium. Taken together, these data suggest that directed mitotic spindle orientation contributes to epicardial EMT and implicates a junctional complex of β -catenin and Numb in the regulation of spindle orientation.

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